Enriched XP-DCs were incubated with PepTivator® HCMV pp65- and CMV IE-1-derived peptide pools, and simultaneously activated with TLR3 agonist poly (I:C) for 6 h.

Activated and antigen-loaded XP-DCs were cocultured with CellTrace™ Dye–labeled autologous CD8+ T cells. Non-loaded XP-DCs were used as control.

After 10 days T cell proliferation was assessed based on cell numbers and the reduction of CellTrace Dye staining intensity.

Specificity of T cell proliferation was analyzed by CMV pentamer staining with the sample stimulated with non-loaded XP-DCs as a control.

Results
CD141(BDCA-3)+ XP-DCs, which were isolated using MACS® technology, express typical DC surface markers such as HLA-DR, CCR7, CD80, CD83 and CD86, and assume a mature DC phenotype after poly (I:C) stimulation (fig. 2B).

Accordingly, maturation markers were upregulated and secretion of typical cytokines, such as GM-CSF, IFN-α, IFN-γ, IFN-λ, TNF-α and several interleukins were detected (fig. 2B).

Materials and methods

Materials

- CD8+ T Cell Isolation Kit, human
- autoMACS Pro Separator
- Cytomegalovirus (CMV) pentamers for pp65 peptide (NLVPMVATV) and IE-1 peptide (VLAELVKQI)
- PepTivator HCMV pp65
- PepTivator CMV IE-1
- RPMI 1640 with 10% FCS
- REAfinity™ Recombinant Antibodies
- MACSQuant® Analyzer 10

Methods

- XP-DCs were enriched from peripheral blood mononuclear cells (PBMC) from HLA-A2.1*CMV- seropositive donors by a two-step procedure consisting of pre-depletion of monocytes, B cells, and T cells, as well as the subsequent positive selection of XP-DCs via CD141(BDCA-3)+ using MACS technology.
- Autologous CD8+ T cells were isolated from PBMCs using the CD8+ T cell Isolation Kit, human.
- Enriched XP-DCs were incubated with PepTivator® HCMV pp65- and CMV IE-1-derived peptide pools, and simultaneously activated with TLR3 agonist poly (I:C) for 6 h.
- Activated and antigen-loaded XP-DCs were cocultured with CellTrace™ Dye–labeled autologous CD8+ T cells. Non-loaded XP-DCs were used as control.
- After 10 days T cell proliferation was assessed based on cell numbers and the reduction of CellTrace Dye staining intensity.
- Specificity of T cell proliferation was analyzed by CMV pentamer staining with the sample stimulated with non-loaded XP-DCs as a control.

Background

The cross-priming capacity of dendritic cells (DCs) is essential for the initiation of effective cytotoxic T cell (CTL) responses against tumors and thus an intense study object in immuno-oncology. In this context, co-cultures of cross-presenting DCs (XP-DCs) and CD8+ T cells in the presence of model antigens with high prevalence in healthy donors, are performed to better understand the underlying immune mechanisms and potential therapeutic applications. However, high-quality automated cell isolation, as well as consistent cell culture and stimulation conditions with easy translation into clinics, are required for successful progression of such studies. The fast and easy automated isolation of fully functional CD8+ T cells and XP-DCs with the autoMACS® Pro Separator, can be easily translated into clinics using MACS® GMP cell isolation and cell culture reagents, in addition to fully closed cell processing platforms such as the CliniMACS® Prodigy.
The cross-presentation capability of activated CD141(BDCA-3)+ XP-DCs, was demonstrated by their capacity to re-stimulate autologous antigen-specific CD8+ T cells in co-culture. T cells restimulated with XP-DCs loaded with pp65- and IE-1-derived peptide pools proliferated in an antigen-specific manner as shown by a 6-fold higher proliferation rate (fig. 3A) and reduced CellTrace™ dye staining intensity (fig. 3B) compared to the sample with non-loaded XP-DCs. Furthermore, antigen specificity was shown by the increased frequency of pp65 peptide (NLVPMVATV)-specific and IE-1 peptide (VLAELVKQI)-specific CD8+ T cells detected by pentamer staining after stimulation with antigen-loaded XP-DCs (fig. 3B).

Figure 2: Flow cytometry analysis of cell surface markers and cytokines using REAfinity antibodies and the MACSQuant Analyzer 10.

Figure 3: Cross-presentation capability of activated CD141(BDCA-3)+ XP-DCs.

Conclusions

MACS® separated CD8+ T cells and CD141(BDCA-3)+ XP-DCs can be successfully used for mixed lymphocyte reaction (MLR) to assess antigen-specific restimulation of CD8+ T cells by XP-DCs.

For more data, download our poster

Fully automated clinical-grade isolation of XP-DCs for the generation of vaccines that stimulate antigen-specific CTL responses

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